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High glutathione-S-transferase (GST) activity was found in hepatopancreas and gill cytosol of the blue crab (Callinectes sapidus) and the digestive gland cytosol of two marine gastropods (Nassarius obsoletus and Cerithium floridanum).

Purification of GST from crab hepatopancreas by Sephadex G-200, DEAE-Sephacel and chromofocusing resulted in the isolation of two isoenzymes with isoelectric points of 5-9 and 5-7 (GST 5-9 and GST 5-7). Antibodies were prepared to these two isoenzymes and the two forms cross-reacted immunologically. The two transferases had similar molecular weights, amino acid compositions, substrate specifities and kinetic parameters.

Crab gill cytosol showed one isoenzyme which reacted with antibodies to GST 5-9 and GST 5-7. The major isoenzyme of N. obsoletus was a basic form while C. floridanum showed a homodimer acidic form. The gastropod GST forms did not react with antibodies to crab GST. The presence of the phenolic antioxidant, butylated hydroxytoluene, in the diet of blue crab or shrimp (Penaeus aztecus) resulted in high hepatic GST activity.

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Marine Invertebrate Glutathione-*S*-transferases: Purification, Characterization and Induction

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*High glutathione-S-transferase (GST) activity was found in hepatopancreas and gill cytosol of the blue crab (*Callinectes sapidus*) and the digestive gland cytosol of two marine gastropods (*Nassarius obsoletus *and* Cerithium floridanum*).*

Purification of GST from crab hepatopancreas by Sephadex G-200, DEAE-Sephacel and chromofocusing resulted in the isolation of two isoenzymes with isoelectric points of 5·9 and 5·7 (GST 5·9 and GST 5·7). Antibodies were prepared to these two isoenzymes and the two forms cross-reacted immunologically. The two transferases had similar molecular weights, amino acid compositions, substrate specifities and kinetic parameters.

Crab gill cytosol showed one isoenzyme which reacted with antibodies to GST 5·9 and GST 5·7. The major isoenzyme of N. obsoletus *was a basic form while* C. floridanum *showed a homodimer acidic form. The gastropod GST forms did not react with antibodies to crab GST. The presence of the phenolic antioxidant, butylated hydroxytoluene, in the diet of blue crab or shrimp (*Penaeus aztecus*) resulted in high hepatic GST activity.*

Glutathione-*S*-transferase (GST) catalyzes the conjugation of glutathione with foreign compounds containing electrophilic centers. It is essential for animals to deal with active electrophiles since they can react with macromolecules controlling cell growth such as RNA, DNA and proteins.

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Many, if not all, chemical carcinogens are electrophiles.[1] Thus, GST plays an important role in detoxifying strong electrophiles having toxic, mutagenic and carcinogenic properties. The enzyme has been found in all animals that have been assayed, including a number of marine invertebrates.[2-5] Our studies were on the GST isoenzymes from a crab (blue crab, *Callinectes sapidus*) and two gastropods (mud snail, *Nassarius obsoletus*, and Florida cerith, *Cerithium floridanum*). Additional studies were carried out to determine the effect of a known GST inducer, butylated hydroxytoluene, on GST activity of *C. sapidus* and shrimp, *Penaeus aztecus*.

We recently carried out the purification and characterization of GST from the hepatopancreas of *C. sapidus*.[4] The purification steps involved Sephadex G-200, DEAE Sephacel and chromofocusing. Similar procedures were used to purify *C. sapidus* gill GST and digestive gland GST of *N. obsoletus* and *C. floridanum*. Antibodies to GST isoenzymes of *C. sapidus* hepatopancreas were prepared by inoculating New Zealand white rabbits with the purified GST isoenzymes. The immunological reactivity was determined by Ouchterlony double-immunodiffusion to observe if the antiserum gave a precipitation line with its own antigen (*C. sapidus* hepatopancreas GST) and against *C. sapidus* gill GST, *N. obsoletus* GST and *C. floridanum* GST.[6] The effect of the antioxidant, 2,6-ditertiary-butyl-4-hydroxytoluene (BHT), on GST activity was determined by adding BHT (1 mg/g food) to prepared food given to *C. sapidus* and the penaeid shrimp (*Penaeus aztecus*).

In earlier studies we found high GST activity in the cytosol of F-cells from the hepatopancreas of the *C. sapidus*.[4] Purification of GST from crab hepatopancreas extracts resulted in the isolation of two isoenzymes with isoelectric points of 5·9 and 5·7 (GST 5·9 and GST 5·7), as determined by analytical isoelectric focusing. The two transferases had similar molecular weights, amino acid compositions, substrate specifications and kinetic parameters (V_{max} and K_m). Also, GST 5·9 and GST 5·7 cross-reacted immunologically. GST 5·9 was a homodimer (monomer molecular weight—22 300) while GST 5·7 was a heterodimer (monomer weights—22 300 and 22 400). The two isoenzymes could also be distinguished by different inhibitor mechanisms with hematin and bromosulfophthalein. GST purified from blue crab gill extracts gave one isoenzyme which reacted with antibodies raised against GST 5·9 and GST 5·7.

A purification of GST from the digestive gland of two gastropods (*Nassarius obsoletus* and *Cerithium floridanum*) was carried out. The major GST isoenzyme of *N. obsoletus* did not bind to DEAE and had an isoelectric point in the basic range (pH 8·1). Minor isoenzymes were in the acid range. *C. floridanum* had no basic transferase isoenzymes and had one acidic homodimer isoenzyme (monomer weight—22 900) with an isoelectric point

TABLE 1

Properties of Crab and Gastropod Glutathione-*S*-Transferases

(The cytosol from blue crabs (*Callinectes sapidus*) hepatopancreas and gastropods (*Nassarius obsoletus* and *Cerithium floridanum*) digestive glands was used to purify glutathione-*S*-transferase (GST) isoenzymes. The substrate for the reported GST activity was 1-chloro-2,4-dinitrobenzene. The cytosol activity is the mean ± standard deviation ($n = 4$).)

Property	*Animal*			
	C. sapidus		N. obsoletus	C. floridanum
	Hepatopancreas	*Gill*		
Number of isoenzymes	2	1	1 (major) 1 (minor) 4 (trace amounts)	1
Cytosol GST activity (μmoles product formed/min-mg protein)	0·8 ± 0·1	0·2 ± 0·05	2·9 ± 0·3	10·7 ± 1·1
GST activity of purified isoenzymes (μmoles product formed/min-mg protein)	222, 182	110	264 479	369
Isoelectric points of isoenzymes	5·9, 5·7	5·9	5·2, 8·1	5·5
Molecular weight of monomers	22 300 (GST 5·9) 22 300, 22 400 (GST 5·7)	—	—	— 22 900
Precipitin reaction with *C. sapidus* (GST 5·9) anti-sera	+	+	—	—

of 5·5. The purified GST from the two gastropods did not react with antibodies to GST 5·9 and GST 5·7 from blue crab hepatopancreas. Thus, there appears to be major differences between the crab GST and mollusks GST that we have examined to date (summarized in Table 1).

GST activity in crab and shrimp showed a significant increase after exposure to the phenolic antioxidant, butylated hydroxytoluene (Table 2). This compound is a strong inducer of hepatic GST activity in mammals.[7] In recent work, we have found very active GST activity in mollusks feeding on certain toxic green algae. Certain unsaturated aldehydes, which account for the toxicity of these algae, should serve as substrates of GST. We hypothesize that toxic unsaturated aldehydes induce specific isoenzymes in mollusks which feed on toxic algae.

TABLE 2

Increase of Glutathione-*S*-Transferase Activity in Crabs and Shrimp After Exposure to 2,6-Ditertiary-Butyl-4-Hydroxy-toluene (BHT)

(A prepared food containing BHT (1 mg/g food) was fed daily for 3 days to brown shrimp (*Penaeus aztecus*) and blue crabs (*Callinectes sapidus*). A second group of animals was fed untreated food. Cytosol from the hepatopancreas was assayed for glutathione-*S*-transferase activity using 1-chloro-2,4-dinitrobenzene as the substrate. Each activity listed in the table is the average of four animals assayed separately ± standard deviation.)

	Glutathione-S-transferase activity (μmoles product formed/min-mg protein)
C. sapidus	
Untreated	0·8 ± 0·2
Fed BHT	3·1 ± 0·5
P. aztecus	
Untreated	0·21 ± 0·07
Fed BHT	0·8 ± 0·2

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REFERENCES

1. Miller, J. A. & Miller, E. C. In *Environmental Carcinogenesis* (P. Emmelot & E. Kriek, eds), Elsevier/North Holland, Amsterdam, pp. 25–50, 1979.
2. Balakaskan, S., Chen, S. & Segaran, M. *Comp. Biochem. Physiol.*, **85B**, 189–92 (1986).
3. James, M. O., Bowen, E. R., Dansette, P. M. & Bend, J. R. *Chem.-Biol. Interactions*, **25**, 321–44 (1979).
4. Keeran, W. S. & Lee, R. F. *Arch. Biochem. Biophys.*, **255**, 233–43 (1987).
5. Tate, L. G. & Herf, D. A. *Comp. Biochem. Physiol.*, **61C**, 165–9 (1978).
6. Ouchterlony, O. *Handbook of Immunodiffusion and Immunoelectrophoresis*, Ann Arbor Science Publishers, Ann Arbor, MI, 215 pp., 1968.
7. Schramm, H., Robertson, L. W. & Oesch, F. *Biochem. Pharmacol.*, **34**, 3735–9 (1985).

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